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# The Effectiveness of Triclosan-Incorporated Plastic against Bacteria on Beef Surfaces†

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## ABSTRACT

Triclosan is a nonionic, broad-spectrum, antimicrobial agent that has been incorporated into a variety of personal hygiene products, including hand soaps, deodorants, shower gels, mouthwashes, and toothpastes. In this study, plastic containing 1,500 ppm of triclosan was evaluated in plate overlay assays and meat experiments as a means of reducing populations of bacteria. Plate overlay assays indicated that the triclosan-incorporated plastic (TIP) inhibited the following organisms: *Brochothrix thermosphacta* ATCC 11509, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922, and several strains of *E. coli* O157:H7. In meat experiment 1, irradiated, lean beef surfaces inoculated with *B. thermosphacta, Salmonella* Typhimurium, *E. coli* O157:H7, or *B. subtilis* were covered with TIP, vacuum packaged, and stored for 24 h at 4°C. Of the organisms tested, only populations of *B. thermosphacta* were slightly reduced. In meat experiment 2, prerigor beef surfaces were inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium, or *B. thermosphacta* incubated at 4°C for 24 h, wrapped in TIP or control plastic, vacuum packaged, and stored at 4°C for up to 14 days. There was a slight reduction in the population of the organisms after initial application with TIP. However, bacterial populations following long-term, refrigerated (4°C), vacuum-packaged storage up to 14 days were not statistically ($P \leq 0.05$) or numerically different than controls. In meat experiment 3, even TIP-wrapped, vacuum-packaged beef samples that were temperature abused at 12°C did not exhibit significant ($P \leq 0.05$) or sustainable reductions after 14 days of 4°C storage. Another study indicated that populations of *E. coli* O157:H7 or *B. thermosphacta* added directly to TIP were not affected after 2 h of refrigerated storage or that the antimicrobial activity could be extracted from the plastic. Additional experiments suggest that presence of fatty acids or adipose may diminish the antimicrobial activity of TIP on meat surfaces. This study demonstrates that while antimicrobial activity is detected against bacterial cultures in antimicrobial plate assays, plastic containing 1,500 ppm of triclosan does not effectively reduce bacterial populations on refrigerated, vacuum-packaged meat surfaces.

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a bis-phenol and a nonionic germicide with low toxicity and a broad spectrum of antimicrobial activity *(7)*. Triclosan has been used as an additive for inhibiting the swarming and growth of *Proteus* spp. on agar medium as well as preventing the growth of enteric bacteria *(2, 6)*. Triclosan alone or in combination with zinc citrate has also been incorporated into toothpastes or mouthwashes and studied as an antiplaque or antigingivitis agent *(3, 7, 10)*. Other reports have determined that triclosan may be an effective antimicrobial agent for reducing bacteria during hand washing *(11)* or as a topical agent for the control of acne *(1)*.

Microban (Huntersville, N.C.) manufactures a wide range of products that contain triclosan. According to the manufacturer, triclosan is added during the extrusion of plastic and fibers. Some products that are manufactured with this technology include cutting boards, garbage bags, carpet, surgical gauze, toothbrushes, toys, and bathroom fixtures (manufacturer's information). There is no published information pertaining to the use of triclosan-incorporated plastic (TIP) against bacteria on meat surfaces. Therefore, this study was conducted to determine the effectiveness of TIP against populations of foodborne pathogenic bacteria as well as bacteria associated with the surfaces of beef. Additional experiments are presented to provide possible reasons for inactivity of TIP on meat surfaces.

## MATERIALS AND METHODS

**Bacterial cultures.** *Brochothrix thermosphacta* ATCC 11509, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC Scott A, *Yersinia enterocolitica* ATCC 23715, *Pediococcus pentosaceous* ATCC FBB61, *Lactobacillus viridescens* ATCC 12706, *Clostridium perfringens* ATCC 13124, and several strains of *E. coli* O157:H7 (ATCC 43895, 35150, 43888, 43889, 43890, 43894, 43895) from the Roman L. Hruska U.S. Meat Animal Research Center (MARC) culture collection were maintained in 75% glycerol at −20°C and propagated for 18 h in Trypticase soy broth (Troy Biologicals, Troy, Mich.) at 26°C for *B. thermosphacta*, 30°C for *P. pentosaceous*, 30°C for *L. viridescens*, and 37°C for all other organisms.

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

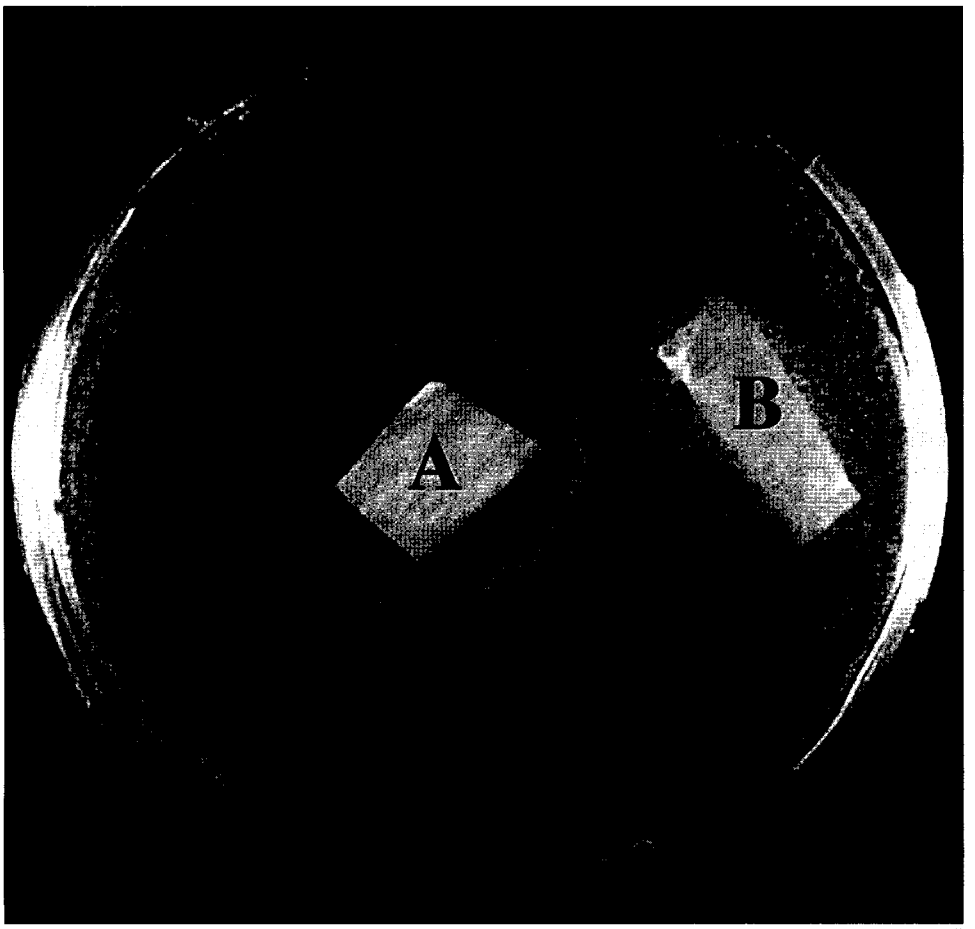


FIGURE 1. *Antimicrobial activity of TIP (A) and control plastic (B) against a lawn of* Salmonella *Typhimurium ATCC 14028.*

A streptomycin-resistant strain of *E. coli* O157:H7 and a nalidixic acid–resistant strain of *Salmonella* Typhimurium were obtained from MARC culture collection and maintained in 75% glycerol at −20°C. The antibiotic-resistant strains of *E. coli* O157:H7 and *Salmonella* Typhimurium were propagated for 18 h in Trypticase soy broth containing 100 μg/ml of streptomycin (Sigma, St. Louis, Mo.) or 250 μg/ml of nalidixic acid (Sigma), respectively, at 37°C for 18 h.

**Plate overlay assay.** Antimicrobial activity of TIP (1,500 ppm of triclosan; Microban) and control plastic samples (no triclosan) against bacteria was determined using a plate overlay assay *(8)*. Briefly, Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates were overlaid with 8 ml of semisoft Trypticase soy agar (0.5% wt/vol agar) seeded with 80 μl of an overnight broth culture of test organism (for list of organisms, see above). The seed density was approximately $1 \times 10^6$ CFU/ml of overlay. Disks or squares of TIP or control plastic were placed directly on the seeded plate. Duplicate plates were scored (±) for zones of inhibition after 24 h of incubation at respective temperatures. Using this procedure, it was determined that antimicrobial activity was detected throughout the TIP; therefore, no preference was made for using one side or another of the TIP in subsequent experiments.

**Meat experiment 1: effect of TIP against bacteria on irradiated, vacuum-packaged beef.** Lean tissues from the outer surfaces of postrigor (24-h postmortem) beef carcasses were obtained from the MARC abattoir, vacuum packaged (Hollymatic, Inc., Model LV10G, Countryside, Ill.) in a standard vacuum-packaging bag (3.2 mil nylon/copolymer bag with oxygen transmission rate at 23°C of 52 $cm^3/m^2$; Hollymatic), and stored at −20°C. Frozen tissues were transported to the Linear Accelerator Facility at Iowa State University (Ames, Iowa) where they were sterilized by electron beam (dose ranged from 32.3 to 39.4 kGy; 10 MEV, 10 kW, 2.80 fpm), kept frozen, and transported back to MARC. Frozen, irradiated tissue was thawed overnight at 5°C and then brought to room temperature (25°C) on the day of the experiment. Overnight cultures of *B. thermosphacta* ATCC 11509, *E. coli* O157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 14029, and *B. subtilis* ATCC 6051 were diluted 1:1,000 in sterile physiological saline (pH 7.0) to obtain a viable cell population of approximately 6 $\log_{10}$ CFU/ml. The cutaneous trunci was trimmed to 10 × 10 cm. The inoculum was inoculated with a 6-cm sterile paintbrush onto the fascia side of the tissue and left undisturbed for 15 min at 25°C. Three inoculated samples were saved for bacterial enumeration at day 0 (see below).

TIP was cut to 10 × 10 cm and placed directly on the inoculated fascia side. All TIP-treated and control tissues (no TIP) were then vacuum packaged in a standard vacuum-packaging bag and held at 4°C for 24 h. Vacuum packaging allowed for direct contact of TIP with the inoculated meat surface. Three replications of the experiment were performed for each organism.

**Meat experiment 2: effect of TIP against bacteria on nonirradiated vacuum-packaged beef.** Lean tissue (cutaneous trunci) from the outer surfaces of prerigor (15-min postexsanguination) beef carcasses was obtained from a local cow/bull processing plant, placed in plastic bags, stored in insulated carriers to prevent

TABLE 1. *Detection of inhibition against test organisms following plate overlay assays with triclosan incorporated plastic (TIP)*

| Organism | Inhibition with TIP[a] |
|---|---|
| *Brochothrix thermosphacta* ATCC 11509 | + |
| *Bacillus subtilis* ATCC 6051 | + |
| *Clostridium perfringens* ATCC 13124 | − |
| *Escherichia coli* O157:H7 ATCC 35150 | + |
| *E. coli* O157:H7 ATCC 43888 | + |
| *E. coli* O157:H7 ATCC 43889 | + |
| *E. coli* O157:H7 ATCC 43890 | + |
| *E. coli* O157:H7 ATCC 43894 | + |
| *E. coli* O157:H7 ATCC 43895 | + |
| *Lactobacillus viridescens* ATCC 12706 | − |
| *Listeria monocytogenes* ATCC Scott A | − |
| *Pediococcus pentosaceous* ATCC FBB61 | − |
| *Salmonella* Typhimurium ATCC 14028 | + |
| *Salmonella* Typhimurium nalidixic acid resistant | + |
| *Shigella flexneri* ATCC 12022 | + |
| *Staphylococcus aureus* ATCC 12598 | + |
| *Yersinia enterocolitica* ATCC 23715 | + |

[a] +, presence of zones of inhibition; −, absence of zones of inhibition. The experiment was performed in duplicate.

rapid cooling, transported to MARC, and used within 2 h of slaughter. The cutaneous trunci was aseptically trimmed to fit onto sterile trays (48 × 30 cm). Overnight cultures of *E. coli* O157:H7 ATCC 43888, *B. thermosphacta* ATCC 11509, or antibiotic-resistant *Salmonella* Typhimurium were diluted 1:1,000 in sterile physiological saline (pH 7.0) to obtain a viable cell population of approximately 6 $\log_{10}$ CFU/ml. A cocktail was also made from equal amounts of diluted cultures of *E. coli* O157:H7 ATCC 43888, 43890, and 43895. Each of the diluted cultures was paintbrush inoculated onto the fascia side of individual pieces of prerigor lean tissue. After 15 min at room temperature, three 25-$cm^2$ samples were excised from the inoculated surfaces and bacterial populations enumerated (see below). The inoculated surfaces were stored at 4°C for 24 h to undergo rigor.

After incubation, the postrigor, inoculated surfaces were trimmed to individual pieces of 7.5 × 7.5 cm. These tissues were prewrapped, envelope fashion, in a 8 × 17-cm piece of TIP or control plastic *(9)*. All tissues were then vacuum packaged as described above and held at 4°C for up to 14 days. Three samples

TABLE 2. *Meat experiment 1—Effect of TIP against bacteria on irradiated vacuum-packaged beef*[a]

| Organism | Control Day 0 | Control Day 1 | TIP Day 1 |
|---|---|---|---|
| *Escherichia coli* O157:H7 ATCC 43895 | 5.54 | 5.26 | 5.54 |
| *Brochothrix thermosphacta* ATCC 11509 | 2.92 | 3.28 | 2.75 |
| *Bacillus subtilis* ATCC 6051 | 3.22 | 2.95 | 3.02 |
| *Salmonella* Typhimurium ATCC 14028 | 5.45 | 5.31 | 5.21 |

[a] Bacterial populations ($\log_{10}$ CFU/$cm^2$) following treatments with control plastic or TIP and vacuum packaged were stored at 4°C for 24 h. The experiment was performed in triplicate.

TABLE 3. *Meat experiment 2—Effect of TIP against bacteria on nonirradiated vacuum-packaged beef*[a]

| Organism | Day 0 | Day 1 | Day 2 | | | Day 5 | | | Day 8 | | | Day 14 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | U | C | TIP | U | C | TIP | U | C | TIP | U | C | TIP |
| *Escherichia coli* O157:H7 ATCC 43889 | 6.48 | 6.27 | 5.72 A | 4.49 B | 4.75 B | 5.88 A | 5.25 A | 5.37 A | 5.70 A | 5.35 A | 5.11 A | 4.83 A | 5.09 A | 5.31 A |
| *E. coli* O157:H7 cocktail[b] | 4.27 | 3.42 | 3.32 B | 3.45 B | 3.83 A | 3.19 B | 3.68 A | 3.69 A | 3.23 B | 3.61 B | 3.21 B | 3.24 A | 3.16 A | 3.01 A |
| *Brochothrix thermosphacta* ATCC 11509 | 4.49 | 4.29 | 4.54 B | 5.73 A | 4.38 B | 5.00 B | 6.09 A | 4.31 C | 5.01 A | 4.95 A | 4.88 A | 5.82 B | 6.65 A | 6.26 AB |
| *Salmonella* Typhimurium ATCC 14028 | 4.36 | 4.26 | 3.95 A | 3.96 A | 4.09 A | 3.91 A | 3.92 A | 3.93 A | 3.84 B | 4.32 A | 4.25 A | 3.71 A | 3.45 A | 3.84 A |

[a] Bacterial populations ($\log_{10}$ CFU/$cm^2$) following treatments with control plastic or TIP and vacuum packaged were stored at 4°C for up to 14 days. U, untreated samples (no control or TIP plastic); C, control plastic (no TIP); TIP, triclosan-incorporated plastic. Different letters indicate statistical difference ($P \leq 0.05$) between treatments, within each row, on a given day.

[b] Cocktail consists of equal amounts of *E. coli* O157:H7 ATCC 43888, 43890, and 43895.

TABLE 4. *Meat experiment 3—Effect of TIP against bacteria on nonirradiated vacuum-packaged beef subjected to temperature abuse*[a]

| Organism | Day 0 | Day 1 | Day 2 | | | Day 5 | | | Day 8 | | | Day 14 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | U | C | TIP | U | C | TIP | U | C | TIP | U | C | TIP |
| *Escherichia coli* O157:H7 ATCC 43889 | 6.26 | 6.23 | 5.94 AB | 6.12 A | 5.82 B | 6.46 A | 6.17 A | 6.06 A | 6.47 A | 6.02 A | 6.55 A | 6.63 A | 6.15 A | 6.78 A |

[a] Bacterial populations ($\log_{10}$ CFU/cm$^2$) following treatments with control plastic or TIP and vacuum packaged were stored at 4°C for 2 days then the temperature was increased to 12°C for up to 14 days. U, untreated samples (no control or TIP plastic); C, control plastic (no TIP); TIP, triclosan-incorporated plastic. Different letters indicate statistical difference ($P \leq 0.05$) between treatments on a given day.

(25 cm$^2$) also were excised from the inoculated postrigor surfaces and bacterial populations enumerated (day 1; see below).

**Meat experiment 3: effect of TIP against bacteria on nonirradiated, vacuum-packaged beef subjected to temperature abuse.** Prerigor, lean, cutaneous trunci was obtained from carcasses as described in experiment 2. An overnight culture of *E. coli* O157:H7 ATCC 43889 was diluted 1:1,000 in sterile physiological saline (pH 7.0) to obtain a viable cell population of approximately 6 $\log_{10}$ CFU/ml. The diluted culture was paintbrush inoculated onto the fascia side of the prerigor tissue and left undisturbed for 15 min at room temperature; three 25-cm$^2$ samples were excised from the inoculated surfaces and bacterial populations enumerated (day 0; see below). The inoculated surfaces were stored at 4°C for 24 h to undergo rigor.

After incubation, the postrigor, inoculated surfaces were trimmed to individual pieces of 7.5 × 7.5 cm. Inoculated postrigor tissues were wrapped in an 8 × 17-cm piece of TIP or control plastic as described in experiment 2, vacuum packaged as described above, held at 4°C for 2 days, and then held at 12°C until day 14. Three 25-cm$^2$ samples also were excised from the inoculated postrigor surfaces and bacterial populations enumerated (day 1; see below).

**Bacterial enumeration.** Following aseptic excision on the specified days, individual 25-cm$^2$ pieces of beef tissue were pummeled for 2 min (Stomacher 400; Tekmar, Inc., Cincinnati, Ohio) in a Sterefil Stomacher bag (Spiral Biotech, Bethesda, Md.) with 25 ml of buffered peptone water (2% BPW, pH 7.0; BBL Microbiology Systems) containing 0.1% Tween 20 (Fisher, St. Louis, Mo.). Each stomachate was serially diluted in BPW, and either spiral plated (Model D Spiral Plater; Spiral Biotech) in duplicate or spread plated in quadruplicate on appropriate selective agar. For the detection of *B. thermosphacta,* stomachates were plated on STAA agar base (Oxoid, Hampshire, England) supplemented with 1.5% glycerol (Sigma), 50 μg/ml of thallium acetate (Sigma), 500 μg/ml of streptomycin sulfate (Sigma), and 50 μg/ml of cycloheximide (Sigma). For the detection of *E. coli* O157:H7 or *Salmonella* Typhimurium, stomachates were spiral plated in duplicate onto sorbitol McConkey agar (Difco Laboratories, Detroit, Mich.) or Rambach agar (Merck, Darmstadt, Germany). Plates were enumerated manually or with the CASBA IV image analyzer (Spiral Biotech) after incubation for 48 h at respective temperatures. The lowest level of detection of organisms was 1.30 $\log_{10}$ CFU/cm$^2$ using spiral plating procedures; samples that were spread plated in quadruplicate were used to detect total number of CFU/cm$^2$.

**TIP experiment 1: direct inoculation of bacteria to TIP.** This experiment was conducted to determine whether TIP exhibited activity against bacterial cultures inoculated directly onto the plastic. A streptomycin-resistant strain of *E. coli* O157:H7 and *B. thermosphacta* were diluted in BPW and 100 μl of each culture was inoculated directly onto six individual disks of TIP or control plastic, cut small enough to fit in a well of a microtiter plate. The microtiter plate was covered and incubated at 4°C for up to 2 h in a pan of distilled water to prevent evaporation. Following removal of all 100 μl with a micropipettor, bacterial populations were enumerated as described above.

**TIP experiment 2: detection of antimicrobial activity of TIP following treatments under various conditions.** Individual 25-cm$^2$ pieces of TIP were subjected to the following treatments to extract antimicrobial activity: (i) water, 25°C, 2 h; (ii) boiling water bath, 5 min; (iii) 0.02 N hydrochloric acid, 25°C, 2 h; (iv) 0.02 N hydrochloric acid, boiling water bath, 5 min; (v) physio-

TABLE 5. *TIP experiment 2—Detection of antimicrobial activity of TIP following extraction and plate overlay assay*

| Extraction conditions | Organism[a] | | | | | |
|---|---|---|---|---|---|---|
| | *Brochothrix thermosphacta* | | *Bacillus subtilis* | | *Salmonella* Typhimurium | |
| | TIP | Supernatant | TIP | Supernatant | TIP | Supernatant |
| Water, 25°C, 2 h | + | − | + | − | + | − |
| Boiling water bath, 5 min | + | − | +[b] | − | +[b] | − |
| 0.02 N HCl, 25°C, 2 h | +[b] | − | + | − | + | − |
| 0.02 N HCl, boiling water bath, 5 min | + | − | + | − | +[b] | − |
| Physiological saline and 0.5% Tween 20, 25°C, 2 h | − | − | − | − | − | − |
| Physiological saline and 0.5% Tween 20, boiling water bath, 5 min | − | − | − | − | − | − |

[a] +, zones of inhibition; −, absence of zones of inhibition.
[b] Only one sample was positive for inhibition.

logical saline and 0.5% Tween 20 (Fisher), 25°C, 2 h; and (vi) physiological saline and 0.5% Tween 20, boiling water bath, 5 min *(9)*.

**TIP experiment 3: detection of antimicrobial activity of TIP following treatments with Tween 20.** Individual 25-$cm^2$ pieces of TIP were treated at 25°C for 2 h in 25 ml of (i) water; (ii) physiological saline; (iii) physiological saline and 0.5% Tween 20 (Fisher); (iv) 0.5% Tween 20; (v) 0.25% Tween 20; (vi) 0.13% Tween 20; (vii) 0.06% Tween 20; (viii) 0.03% Tween 20; and (ix) 0.015% Tween 20 *(9)*. Duplicate plate overlay assays were performed with 1 × 1-cm pieces of treated TIP or 20 μl of supernatant.

**TIP experiment 4: detection of antimicrobial activity of TIP following treatments with sponge samples taken from lean or adipose beef surfaces.** Lean and adipose beef carcass tissues were obtained from prerigor beef carcasses, vacuum packaged, stored at 4°C for 24 h, and frozen at −20°C until needed. On the day of the experiment, tissues were thawed at 4°C and allowed to equilibrate to room temperature. Individual sterile sponges (NASCO, Ft. Atkinson, Wis.) were premoistened in 25 ml of sterile physiological saline and the liquid expressed before swabbing. Using a premoistened sponge and a stainless steel template, samples from each tissue type were taken as follows: 100 $cm^2$ of the lean or adipose surfaces were swabbed 10 times in a horizontal direction, the sponge flipped, and swabbed 10 times in a vertical direction. The sponge was returned to the NASCO bags, stomached for 2 min (Model 400 Stomacher; Tekmar), and the remaining liquid expressed from the sponge (approximately 15 ml). The expressed liquid was transferred to a 50-ml conical centrifuge tube. Individual 25-$cm^2$ pieces of TIP were cut and added to expressed liquid at 25°C for 2 h. Duplicate plate overlay assays were performed with approximately 1 × 1-cm pieces of treated TIP.

TABLE 6. *TIP experiment 3—Detection of antimicrobial activity of TIP following extraction and plate overlay assay*

| Extraction conditions (25°C, 2 h) | Organism[a] | | | |
|---|---|---|---|---|
| | *Brochothrix thermosphacta* | | *Salmonella* Typhimurium | |
| | TIP | Supernatant | TIP | Supernatant |
| Water | + | − | + | (+) |
| Physiological saline | + | − | + | − |
| Physiological saline and 0.5% Tween 20 | − | − | − | − |
| Physiological saline and 0.25% Tween 20 | − | − | − | − |
| Physiological saline and 0.13% Tween 20 | − | − | − | − |
| Physiological saline and 0.06% Tween 20 | + | − | + | − |
| Physiological saline and 0.03% Tween 20 | + | − | ND | − |
| Physiological saline and 0.015% Tween 20 | + | − | + | − |
| No treatment | + | NA | + | NA |

[a] +, zones of inhibition; (+), slight zones of inhibition; −, absence of zones of inhibition; ND, not determined; NA, not applicable.

**Calculations and statistical analyses.** After enumeration in meat experiments 1, 2, and 3, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ CFU/$cm^2$. Least-squares means of bacterial populations ($\log_{10}$ CFU/$cm^2$) were calculated from three samples for days 0 and 1 and six samples for all other days for meat experiments 2 and 3. Analysis of variance was performed using the General Linear Models procedure of SAS (SAS for Windows, release version 6.12; SAS Institute, Inc., Cary, N.C.). Inoculum counts were used as a covariant to normalize data between treatment replications. Statistical significance was defined as $P \leq 0.05$, unless otherwise noted.

## RESULTS AND DISCUSSION

The effect of TIP against bacteria was determined in a plate overlay assay (Figure 1). Of the organisms assayed,

TABLE 7. *TIP experiment 4—Detection of antimicrobial activity of TIP following treatments with sponge samples taken from lean or adipose beef surfaces (25°C, 2 h)*

| Extraction conditions | Organism[a] | |
|---|---|---|
| | *Brochothrix thermosphacta* | *Salmonella* Typhimurium |
| Sponge control (no sponge sample from beef surface) | 4/4 | 4/4 |
| Sponge sample from lean surface | 3/4 | 4/4 |
| Sponge sample from adipose surface | 1/4 | 2/4 |

[a] Number of samples/number of replications exhibiting zones of inhibition in plate overlay assays.

TIP inhibited 13 of the 17 strains tested (Table 1). TIP inhibited both gram-negative and gram-positive organisms, as indicated by complete zones of inhibition on the plates; the control plastic inhibited none of the organisms, as indicated by no zones of inhibition on the plates.

For meat experiment 1, populations of *B. thermosphacta* ATCC 11509 on irradiated lean beef carcass tissue, covered with TIP, vacuum packaged, and stored at 4°C for 24 h were reduced slightly compared with controls. Populations of *E. coli* O157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 14028, and *B. subtilis* ATCC 6051 were not reduced (Table 2).

For meat experiments 2 and 3, various organisms were inoculated onto lean beef carcass tissue, covered with TIP, vacuum packaged, and stored at 4°C for up to 14 days. Populations of *E. coli* O157:H7 ATCC 43889, *B. thermosphacta* ATCC 11509, *Salmonella* Typhimurium ATCC 14028, and a cocktail of *E. coli* O157:H7 ATCC 43888, 43890, and 43895 were not reduced by TIP by day 14 (Table 3). Shifting samples to 12°C was done to mimic temperature abuse situations that might occur in the food distribution chain *(9)*. In this case, TIP did not reduce populations of *E. coli* O157:H7 after storage at 12°C (Table 4).

Additional plastic experiments demonstrated that direct inoculation of TIP with bacterial cultures, and storage at 4°C for up to 24 h did not result in reductions in bacterial populations (TIP experiment 1; data not presented). It was also demonstrated that antimicrobial activity was not detected in the supernatant following extraction of TIP under various conditions (Table 5). However, in TIP experiment 3, slight zones of inhibition were detected against *Salmonella* Typhimurium when TIP was extracted in water for 2 h at room temperature (Table 6). Based on these preliminary studies, it appears that antimicrobial activity was not leaching significantly from TIP.

In TIP experiment 2, the antimicrobial activity of TIP following treatments with physiological saline and 0.5% Tween 20 was not detected. In TIP experiment 3, TIP was treated with different concentrations of Tween 20, and the remaining activity was determined against *Salmonella* Typhimurium or *B. thermosphacta*. In this experiment, a concentration of Tween 20 between 0.5% and 0.13% affected antimicrobial activity of TIP after 2 h at room temperature (Table 6). The loss of antimicrobial activity was not detected when TIP was left untreated or was treated with water, physiological saline, or water containing Tween 20 at a concentration between 0.15% and 0.06%. It is possible that fatty acids (Tween 20 contains lauric, myristic, palmitic, and stearic acids; Sigma Catalog) may be interfering with the active ingredient such that antimicrobial activity is diminished. To further investigate this hypothesis, sponge samples were obtained from either lean or adipose beef surfaces, and TIP was treated with these mixtures for 2 h at room temperature. The findings from this experiment indicate that TIP treated with sponge samples taken from adipose surfaces exhibited less antimicrobial activity than TIP treated with control sponges or sponge samples taken from lean surfaces (Table 7). It should be noted that lean surfaces did exhibit some visible adipose. These findings, coupled with the previous experiment, suggest that fatty acids or components associated with adipose tissues may interfere with antimicrobial activity from TIP.

Previous studies have demonstrated not only that incorporation of antimicrobials into plastics *(9)* or edible films *(4, 5)* exhibit activity in plate overlay assays, but also that these coatings can effectively reduce bacterial populations on meat surfaces. Based on the conditions described in the present study, it appears that when plastic containing 1,500 ppm triclosan is combined with vacuum packaging and refrigerated storage, bacteria are not sufficiently reduced on meat surfaces. The possible interaction of the antimicrobial with adipose components may be responsible for this inactivity against bacteria on meat surfaces.

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